Low-intensity pulsed ultrasound (LIPUS) exposure has been used as a treatment procedure for healing the fractures both in animal models and as well as in clinical studies. LIPUS shortens the normal fracture-healing process of the tibia and radius, a fact pointed out by several clinical studies [1–3]. Animal studies have also shown that LIPUS exposure increases maximum resistance to failure and stiffness in diaphyseal fractures in rats [4–6]. Recently, Azuma et al. [7] pointed out that LIPUS hurries up the rat femoral fracture healing. Mayr et al. [8] reported that LIPUS exposure induced healing of delayed unions at a rate of 91% and healing of nonunion at a rate of 86%. In vitro, the molecular pathway that mediates this action is gradually being clarified. LIPUS exposure increases aggrecan messenger RNA levels and proteoglycan synthesis in chondrocyte cultures [9, 10] and calcium incorporation in cultured bone cells [11] and modulates transforming growth factor synthesis and adenylylate cyclase production in osteoblasts [12]. It is still not very clear which is the process based upon LIPUS exposure initiates bone healing in nonunion. The aim of this study is to establish whether LIPUS exposure initiates bone healing in rat nonunion fracture models.

LIPUS is a form of mechanical power that can be transferred into living tissue as acoustic intensity waves. The micromechanical strains which these intensity waves are producing in living tissue can become biochemical occurrence at the cellular level [13–15]. This may occur through several possible mechanisms. The compression of micro-bubbles or cavitations and acoustic streaming could have a direct effect on cell membrane permeability [11,16,17]. Cation channels can be activated by the effect of mechanical pressure at the cell surface [18]. Ultrasound may also affect the attachment of the cytoskeleton to the extracellular matrix [19]. Wang et al. [6] pointed out that LIPUS stimulation enhanced the mechanical properties of the healing callus. We also regard LIPUS as a form of mechanical stress, which initiates bone healing in rat nonunion fracture models.

**Experimental part**

50 Wistar rats, 6 months old from the Animal House of the “Pius Branzeu” Center for Laparoscopic Surgery and Microsurgery with an average weight of animals at surgery of 300g, were used in this experiment. The bone augmentation materials were implanted bilaterally in the medullar cavity of the femur of each animal. For the entire experimental period of time the animals will be kept two or three in a cage with an unlimited supply of fresh water and rodent pellets.

Anesthesia of the animals is achieved with Isofluran in concentration of 3% and O2 at 1L/min, for induction in the anesthesia chamber. After this first procedure the animal is connected throw a facemask to an open breath circuit of anesthesia which inflows Isofluran at 1% and O2 at 1L/min. The hindquarter on the experimental side is shaved, the periosteum is made throw a 3 cm longitudinal incision of anesthesia which inflowsIsofluran at 1% and O2 at 1L/min. The hindquarter on the experimental side is shaved, and the entire dorsal aspect of the animal is disinfected. Surgery is performed under sterile conditions. Access to the periosteum is made throw a 3 cm longitudinal incision of the skin on the lateral part of the thigh region, in the cranial third, followed by the blunt dissection of the quadriceps muscle with exposure of proximal diaphysis of femoral bone. All the maneuvers are performed under the microscope and the tissues are handled with microsurgical instruments to prevent as much as possible the tissue destruction.
Closure of the surgical site is undertaken with Monosyn 5.0 continuous suture in 2 planes of the muscles and separated stitches of the skin with Prolene 5.0 thread. Antibiotic prophylaxis during the surgery is performed with Cefazolin + Gentamicin to prevent any infection with any Staph, strep, Gram(-) bacilli or anaerobes. Postoperatively all the animals receive 5 days long analgesic medication with Buprenorphine (fig. 1).

The tomography reconstruction was performed by means of the common filtered back-projection method. For 3D visualization, data volumes were rendered directly without decomposing them into geometric primitives. A commercial software - VGStudio MAX - was used to generate 3D images and to visualize the distribution in 3D of different constituents.

**Results and discussions**

The samples where the Low Intensity Pulsed Ultrasound was used for Bone Augmentation revealed a good quantity and quality of a new bone formation in the area of the induced defect (fig. 4). Also the amount of the remaining augmentation materials is smaller compared with the samples where the ultrasound technology was not employed (fig. 5).

VGStudio Max software gives a comprehensive 3D visualization of the reconstructed specimen, allowing the segmentation of the grey histogram, in order to visualize...
only the phases of interest in the imaged volume. It allows a direct view of three orthogonal axis (Axial, Sagittal and Frontal), together with the 3D image that can be rotated or slices in any direction for a good visualization of the morphology of the reconstructed specimen.

Conclusions

The specific mechanism by which low intensity pulsed ultrasound technology accelerate bone healing remains unknown; however, in terms of the physical mechanism, this technology may exert a mechanical force on cell in soft tissue at the fracture gap.

Evaluation of the bone grafting material/bone interface with noninvasive methods such as microCT using the synchrotron radiation could act as a valuable procedure that can be used in the future for usual research procedures.

The authors want to acknowledge the support of: “This paper was published under the frame of Young Researchers Grant No.15250/19.12.2012

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Manuscript received: 26.01.2014